Biocriteria Trawl Metrics for Groundfish and Epibenthic Marine Invertebrates as a Measure of Environmental Stress and Recovery

Charles M. Eaton
Bio-Marine Enterprises

Introduction

The idea behind the development of biological criteria (biocriteria) is to measure the impact of environmental stress directly on the biological populations present at the test sites. Index scores are generated by comparing these population parameters with those derived from sampling reference populations living under the same physical conditions in a cleaner environment. The hope is to develop more economical, integrative and ecologically relevant measures of environmental stress useful for both initial assessment and for tracking environmental cleanup. There is already a long history of developing these metrics for invertebrate infaunal populations from sediment samples.

This paper will discuss efforts to develop metrics for another biological assemblage—bottomfish and large epibenthic marine invertebrates—sampled with the 7.6-m SCCWRP Trawl (Mearns and Allen 1978). The year 2000 was the fifth year of sampling this biological assemblage[1].

Trawl samples were first conducted in 1993 (Figure 1) when the contaminated Hylebos Waterway in Tacoma was compared to the adjacent less-contaminated Blair Waterway for reference (Eaton and Dinnel 1993). In 1994, the contaminated Thea Foss Waterway was compared to a more natural reference condition in Quartermaster Harbor (Eaton 1994, 1995). In September 1997, two strata in Sinclair Inlet at Bremerton adjacent to the Puget Sound Naval Shipyard were compared to a combination of reference strata in Upper Port Orchard and Quartermaster Harbor (Figure 2) (Eaton 1997, 1998). In September 1999, test sites in Dyes Inlet and Liberty Bay were added to the Sinclair Inlet test site, and a "farfield" stratum III in Sinclair inlet was added adjacent to the "nearfield" stratum III first sampled in 1997. Sinclair Inlet and the reference strata were once again sampled in September 2000 for interannual variation studies. In summary, test sites in the Hylebos and Thea Foss Waterways of Tacoma were sampled in 1993 and 1994, and in Sinclair Inlet in 1997, 1999, and 2000. Dyes Inlet and Liberty Bay (Poulsbo) were sampled in 1999. These test sites were compared to reference strata from the Blair Waterway in Tacoma and from Quartermaster Harbor and upper Port Orchard. All trawls after 1993 were conducted in late August and September.

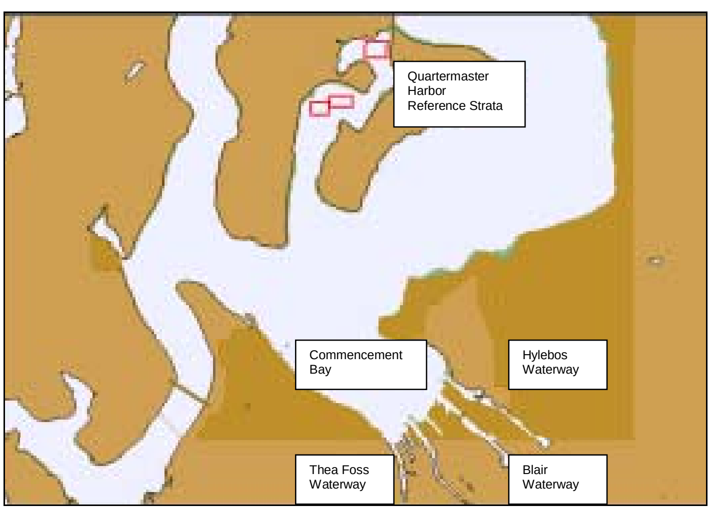

Figure 1. Test sites in the Hylebos Waterway (1993) and the Thea Foss Waterway (1994) and references.

[1] Supported by funds from the Estuarine/Near Coastal Bioassessment/Biocriteria Project, U.S. Environmental Protection Agency, Washington D.C.

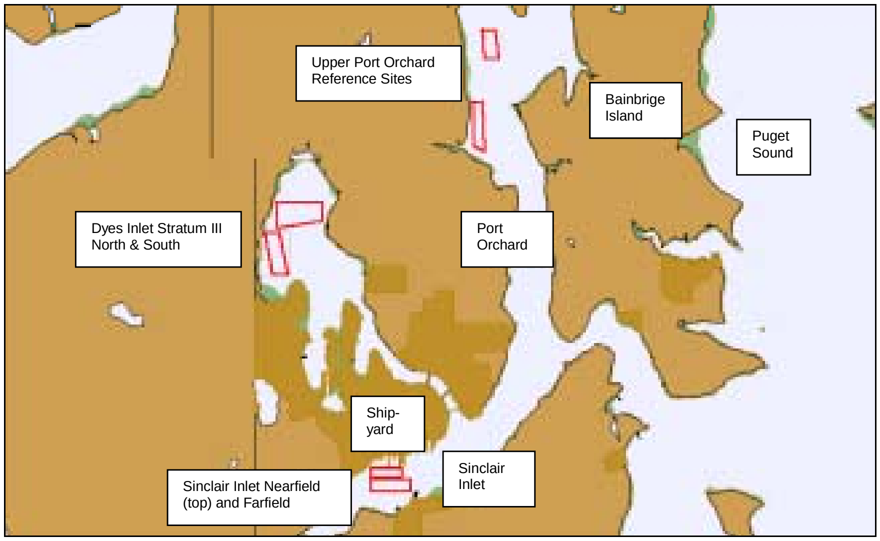

Figure 2. Stratum III test sites in Sinclair and Dyes Inlets. Reference strata in upper Port Orchard.

Study Design

The strata themselves are defined by physical characteristics such as depth, sediment grain size, dissolved oxygen and salinity. Stratum III, for example—whether in Sinclair Inlet or Quartermaster Harbor—is 10 to 13 meters deep (MLLW) with a fine silt sediment of 70-90% fines, and comparable September salinity (31 to 32 ppt.) and dissolved oxygen (6.7 to 7.8 mg/liter). Stratum IV, on the other hand, is deeper with a slightly coarser substrate. By comparing strata of comparable physical characteristics, one avoids the problem of comparing apples to oranges…or in this case shallow water fauna to deeper fauna, or fauna from a silt substrate to fauna from a coarser substrate. Comparison of test sites to physically comparable reference strata should highlight environmental stress rather than habitat differences.

Five to 10 replicate trawls were taken within each stratum, thereby defining test and reference conditions as a range of values best displayed graphically as boxplots. The trawl stations were located randomly using the navigation software (Nobeltec's Visual Navigation Suite) and MS Excel's random number generator. Each trawl was 370 meters in length and was pulled at an overall speed of 2.4 to 2.6 knots, as determined by the DGPS. The trawl, at this speed, takes just under five minutes to complete. The catch was then sorted by species into tubs of running seawater, counted, weighed, and returned live on station. Seven to 10 fully processed trawls could be conducted this way in a single day.

In 1993 and 1994, the 3-meter Gunderson beam trawl (Gunderson and Ellis 1986) was used in addition to the standard 7.6-meter SCCWRP trawl. This trawl was much more effective at catching all invertebrates as well as sculpins, sanddabs, eelpouts and gobies (Eaton 1994).

In 1999, another version of the 7.6-m. SCCWRP trawl was tested against the standard version used in 1993 and 1994. This modified version used by the National Marine Fisheries Service has the same mesh-size and headrope and footrope dimensions, but has larger metal doors, a higher rise, and substitutes a footrope of half-inch poly-wire rope for the fiber rope and chain used on the standard version. A detailed comparison of 10 trawls made with each version (20 total), in two different areas, highlighted the ability of the modified high-rise trawl to significantly increase the catch of those fish and invertebrates sampled with the Gunderson beam trawl (Appendix 1). At the same time, both trawls were equally effective at catching flatfish, and there were no significant differences in total fish or flatfish abundance and biomass between the two trawl types. By switching to the modified version of the SCCWRP trawl in 1999, the use of the Gunderson beam trawl could be eliminated, providing a great savings in precious boat time. Switching to the modified version also standardized our sampling gear to that used by the National Marine Fisheries Service (NMFS) in their national surveys.

Results

Fish Biomass

Biomass measurements incorporated in the second year of sampling added greatly to the power of the trawl metrics. By weighing each species as a group, usually divided into adults and subadults, biomass and average weights could be obtained quickly and economically. Boxplots of the replicate trawls from 1994 through 2000 show the consistent pattern of reduced biomass, compared to reference, at known sites of contamination—two strata from the Thea Foss Waterway in Tacoma in 1994, two strata from Sinclair Inlet in 1997, and Sinclair Inlet Stratum III Nearfield in 1999 and 2000 (Figure 3).

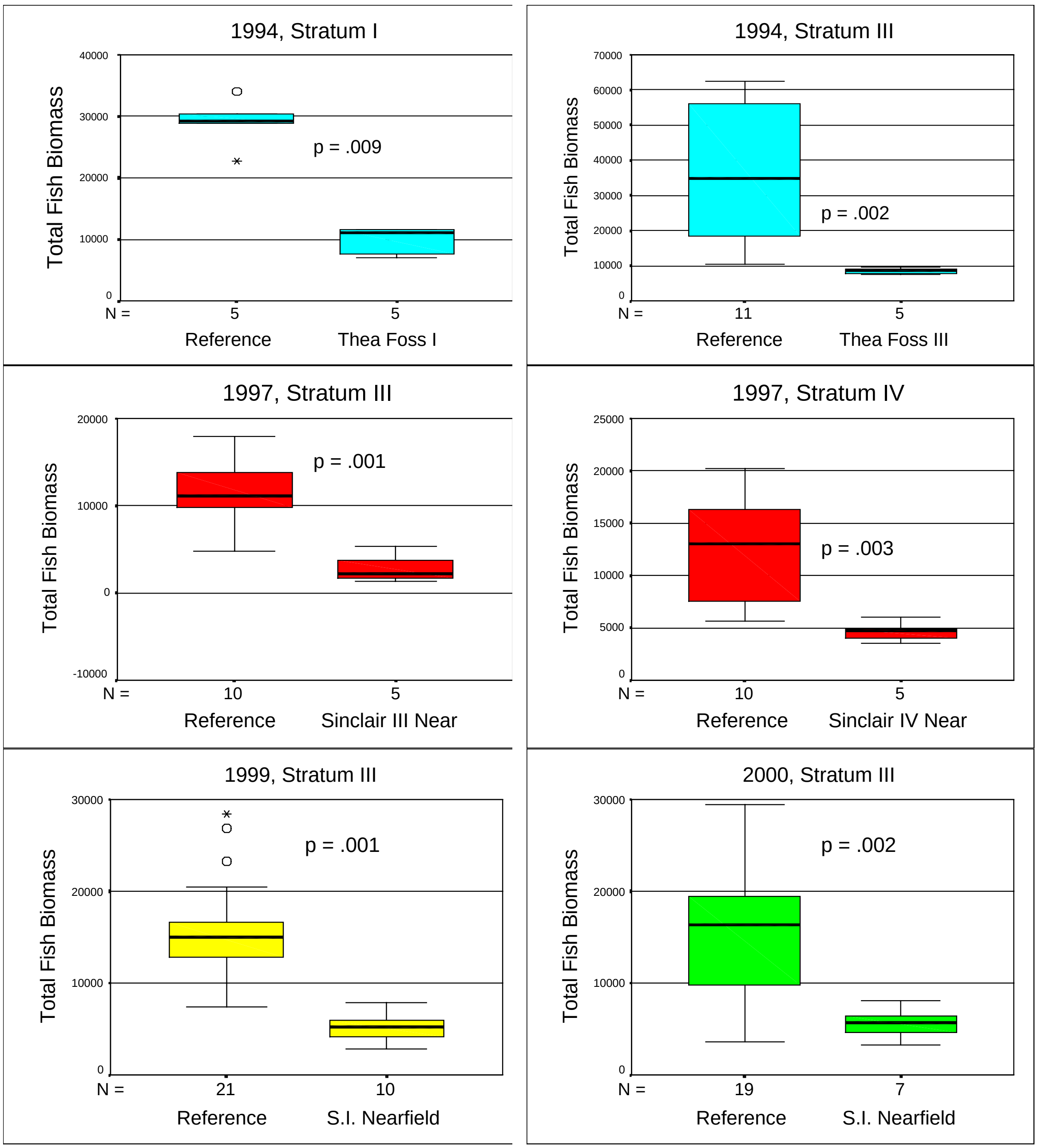

Figure 3. Total fish biomass, 1994 through 2000.

Box plots were chosen because of their ability to visually represent the central tendency of a range of data generated by replicate sampling. The box itself, called the interquartile range (IQR), represents 50% of all cases and extends from the 25^{th} to the 75^{th} percentile with the dark horizontal line within the box representing the median value (50^{th} percentile). The vertical lines (whiskers) are drawn to the largest and smallest values that are outside the box but within 1.5 box lengths, and represent the range of data not considered to be outlying or extreme. Outliers (o) lie within 1.5 to 3 box lengths from the upper and lower edges of the box, and extreme values (*) are more than three box lengths from the upper and lower edges. The central tendency is represented by the median (the horizontal line within the box), the spread or variability by the length of the box and whiskers, and the symmetry of the spread by the position of the median line within the box. If the median line is closer to the bottom of the box than to the top, there is a tail toward the larger values (positive skewness) or vice versa, with the length of the tail shown by the length of the box and the length of the whiskers and the outlying and extreme values. N (number) on the horizontal axis is the number of replicate trawls, and biomass is in grams. The significance of the difference between the boxes is the p-value, with $p < .05$ considered a significant difference. The biocriteria are often defined visually by the boxplots themselves, with the lower edge of the box (the 25^{th} percentile) for the displayed metric variable from the reference areas serving as the goal or criterion.

Biomass and the Nearfield-Farfield Comparison

In 1999, the farfield stratum III in Sinclair Inlet was added adjacent to the nearfield stratum first sampled in September 1997 (Figure 2). The farfield stratum has the same depth range and physical characteristics as the nearfield stratum but is farther from the primary source of pollution—the Puget Sound Naval Shipyard on the north shore of Sinclair Inlet. The *2000 Puget Sound Update*, page 60, illustrates the reduction in metals contamination of the sediments along this north-south gradient proceeding away from the shipyard in Sinclair Inlet. This farfield stratum is represented below by the boxplot in the middle of each graph.

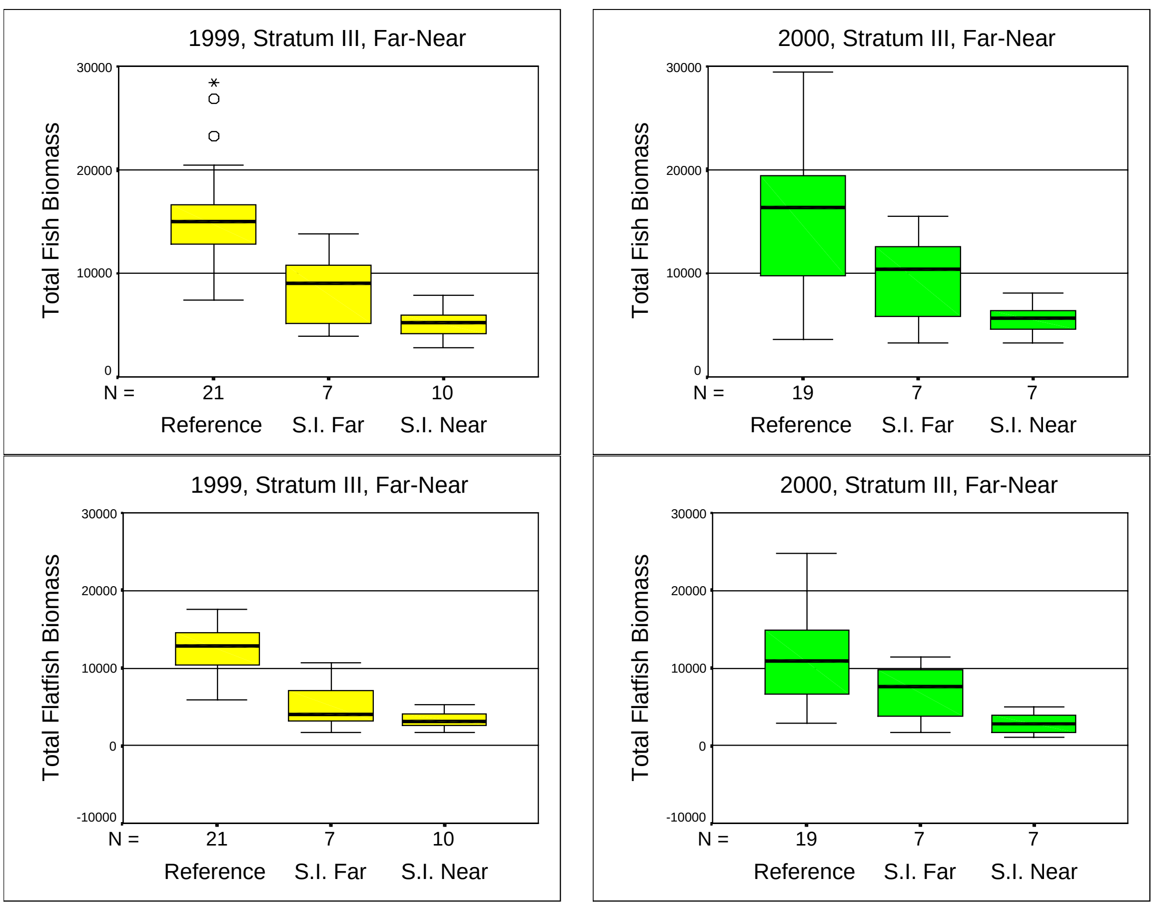

Figure 4. Total fish biomass and flatfish biomass for Sinclair Inlet, reference-farfield-nearfield, 1999-2000.

Total fish biomass (the top two graphs) and flatfish biomass (the bottom two graphs) were remarkably consistent between sampling years September 1999 and September 2000, with the reference strata always significantly higher than the Sinclair Inlet nearfield stratum, while the farfield stratum is consistently intermediate between the reference strata and the more contaminated nearfield stratum.

Sensitive and Tolerant Species—The Sensitivity Index

Some species seem almost unaffected by contamination. These tolerant species may even show the opposite trend displayed by total fish biomass and flatfish biomass with the highest biomass and abundance recorded from the most impaired sites. Examples of tolerant species include the purple cancer crab, *Cancer gracilis*, Pacific tomcod, and staghorn and roughback sculpins (Figure 5). Sensitive species, such as starry flounder, were consistently reduced at the contaminated test sites compared to the reference sites.

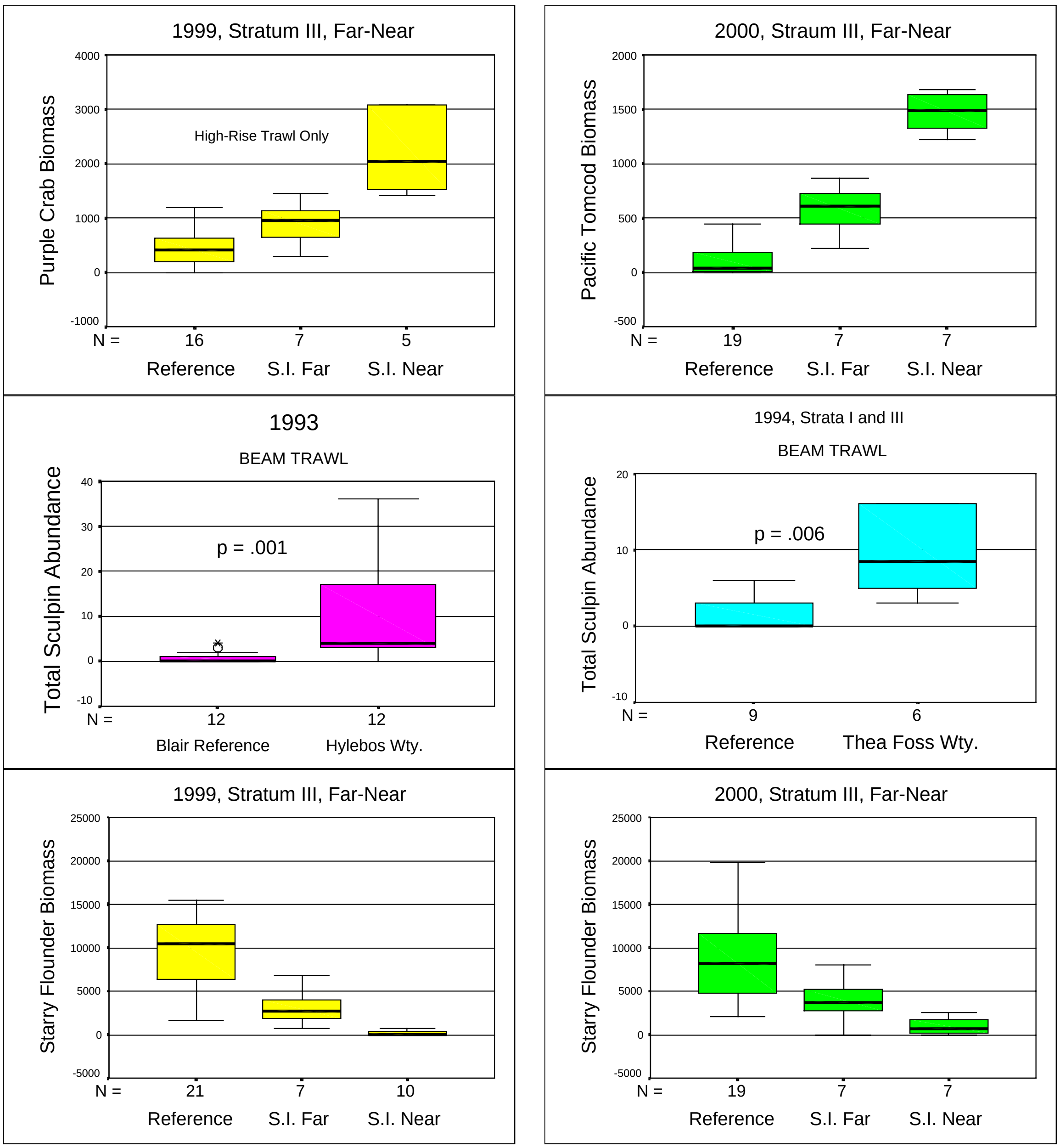

Figure 5. Tolerant species often show enhanced biomass and abundance at contaminated test sites. Sensitive species, such as starry flounder, suffer reductions in biomass at contaminated sites.

A good characterization of sensitive and tolerant species has been gleaned from the five years of trawling contaminated hotspots and reference areas of the same physical parameters (Appendix 2). For a species to be labeled as sensitive or tolerant, it must display a consistent pattern over the five-year period. For each year of sampling, all the variables from each stratum are compared to the combination of reference strata using the non-parametric Mann-Whitney U-test (Figure 6). This test, which is sensitive to the medians and rankings displayed by the boxplots, must show consistency over time for a variable to be included in the sensitive or tolerant categories. Figure 7 shows examples of a sensitive variable, starry flounder biomass, and a tolerant variable, adult purple crab abundance, over the years of sampling. Starry flounder biomass was not available for 1993 since no biomass was recorded that year. Tolerant species are also found in reference areas, and occasionally in great numbers. *Cancer gracilis* populations increased in the reference areas in 2000 to the point where this tolerant species abundance was statistically indistinguishable between the Sinclair Inlet test sites and the reference condition (Figure 7). The purple crab, however, still meets the definition of a tolerant species over time by showing increased (p<.005) or no significant differences in abundance and biomass at contaminated test sites compared to reference strata.

2000, Reference (PO&QMH) vs. Sinclair Inlet Nearfield, HIGH-RISE OT										**BIOMASS**				
N = 19 Reference, N = 7 S.I. Near														
Test Statistics	BB.EELPT	BONY.FSH	BONY.RND	C.GRC.A	C.GRC.S	CHOND	CRUST	CUC.PIP	CUC.TOT	DENDRONT	E.SOL.A	E.SOL.S	E.SOL.T
Mann-Whitney U	49.0	15.0	39.0	34.5	51.0	17.5	36.0	30.0	27.5	57.0	26.0	44.0	15.0
Asymp. Sig. (2-tailed)	0.142	0.003	0.112	0.064	0.360	0.003	0.078	0.024	0.024	0.577	0.019	0.184	0.003
Highest Median	REF	REF	NEAR	NEAR	NEAR	REF	NEAR	REF	REF	REF	REF	REF	REF
Test Statistics	ES.A.AVG	EVASTER	FF.A.AVG	FISH.TOT	FL.FSH.A	FL.FSH.S	FLAT.FSH	GOBY.TOT	HERRING	HIPPOLYT	LUIDIA	MIDSHIP	NUDIBRAN
Mann-Whitney U	43.0	66.5	40.0	12.0	4.0	51.0	3.0	25.5	40.0	22.5	45.5	22.0	14.0
Asymp. Sig. (2-tailed)	0.373	1.000	0.126	0.002	0.000	0.370	0.000	0.018	0.102	0.009	0.100	0.010	0.002
Highest Median	NEAR		REF	REF	REF	REF	REF	NEAR	REF	NEAR	REF	NEAR	NEAR
Test Statistics	P.BF.FF	P.DANAE	PARASTIC	PIS.BREV	PRCH.TOT	RGHB.SCL	RK.SOL.A	RK.SOL.S	RK.SOL.T	RND.FI.A	RND.FI.S	RND.FISH	SCL.TOT
Mann-Whitney U	1.0	40.0	53.0	57.0	63.0	63.0	51.5	48.5	57.0	44.0	7.0	50.0	50.0
Asymp. Sig. (2-tailed)	0.000	0.124	0.411	0.099	0.840	0.812	0.372	0.295	0.583	0.193	0.001	0.340	0.340
Highest Median	REF	NEAR	REF	NEAR	NEAR	REF	NEAR	REF	NEAR	REF	NEAR	REF	REF
Test Statistics	SEASTARS	SEN.GM3	SEN.IND3	SF.A.AVG	SHIN.TOT	SHINER.A	SHINER.S	SN.SOL.A	SN.SOL.S	SN.SOL.T	SNAKE.A	SNAKE.S	SNK.TOT
Mann-Whitney U	57.5	0.0	0.0	38.0	63.0	60.0	59.5	44.0	49.5	55.0	62.0	53.5	66.5
Asymp. Sig. (2-tailed)	0.553	0.000	0.000	0.499	0.840	0.707	0.621	0.184	0.326	0.506	0.792	0.442	1.000
Highest Median	REF	REF	REF	REF	NEAR	NEAR	NEAR	NEAR	REF	NEAR	REF	REF	
Test Statistics	SOLASTER	SPEC.DAB	STAGHORN	STARRY.A	STARRY.S	TCOD.TOT	TOL.GM3	TOMCOD.A	TOMCOD.S	WH.SP.GR			
Mann-Whitney U	56.5	60.5	50.0	2.0	63.0	0.0	1.0	0.0	1.0	38.0			
Asymp. Sig. (2-tailed)	0.357	0.729	0.340	0.000	0.544	0.000	0.000	0.000	0.000	0.003			
Highest Median	NEAR	REF	REF	REF	REF	NEAR	NEAR	NEAR	NEAR	NEAR			

Figure 6. Sinclair Inlet Nearfield vs. Reference, 2000. Results are color-coded for degrees of significance. Significant differences (p<.005) are in yellow and outlined.

Table 1. Examples of how sensitive and tolerant variables are defined over time using the Mann-Whitney U-test and SPSS 7.0 statistical software.

STARRY FLOUNDER BIOMASS							
		Mann-Whitney U					
YEAR	HIGHEST MEDIAN	2-Tailled Signif.	N	Comparison	Reference	Sampling Gear	Comments
2000	Farfield Reference	.034	7/7	Farfield / Nearfield, Stratum III	Farfield	Otter Trawl	
2000	Reference	.000	19/7	Reference / S.I. Stratum III, Nearfield	P.O. and QMH	Otter Trawl	
2000	Reference	.014	19/7	Reference / S.I. Stratum III, Farfield	P.O. and QMH	Otter Trawl	
1999	Farfield Reference	.001	7/10	Farfield / Nearfield, Stratum III	Farfield	Otter Trawl	
1999	Reference	.000	21/10	Reference / S.I. Stratum III, Nearfield	P.O. and QMH	Otter Trawl	
1999	Reference	.002	21/7	Reference / S.I. Stratum III, Farfield	P.O. and QMH	Otter Trawl	
1997	Reference	.002	10 / 5	Reference / S.I. Stratum III, Nearfield	P.O. and QMH	Otter Trawl	
1997	Reference	.039	10 / 5	Reference / S.I. Stratum IV, Nearfield	P.O. and QMH	Otter Trawl	
1994	Reference	.009	5/5	Reference / T.F. Stratum I	QMH	Otter Trawl	
1994	Thea Foss Wty.	.455	11/5	Reference / T.F. Stratum III	QMH	Otter Trawl	
1993	Not available	NA	11/12	Reference / Hylebos Waterway	Blair Wty.	Otter Trawl	
ADULT PURPLE CANCER CRAB (C. gracilis) ABUNDANCE							
		Mann-Whitney U					
YEAR	HIGHEST MEDIAN	2-Tailled Signif.	N	Comparison	Reference	Sampling Gear	Comments
2000	Farfield Reference	.701	7/7	Farfield / Nearfield, Stratum III	Farfield	Otter Trawl	High-Rise OT
2000	Sinclair Inlet	.202	19/7	Reference / S.I. Stratum III, Nearfield	P.O. and QMH	Otter Trawl	High-Rise OT
2000	Sinclair Inlet	.182	19/7	Reference / S.I. Stratum III, Farfield	P.O. and QMH	Otter Trawl	High-Rise OT
1999	S. I. Nearfield	.004	7 / 5	Farfield / Nearfield, Stratum III	Farfield	Otter Trawl	High-Rise OT
1999	Sinclair Inlet	.001	16/5	Reference / S.I. Stratum III, Nearfield	P.O. and QMH	Otter Trawl	High-Rise OT
1999	Sinclair Inlet	.020	16/7	Reference / S.I. Stratum III, Farfield	P.O. and QMH	Otter Trawl	High-Rise OT
1997	Reference	.541	10 / 5	Reference / S.I. Stratum III, Nearfield	P.O. and QMH	Otter Trawl	Standard OT
1997	Sinclair Inlet	.002	10 / 5	Reference / S.I. Stratum IV, Nearfield	P.O. and QMH	Otter Trawl	Standard OT
1994	Thea Foss Wty.	.050	3 / 3	Reference / T.F. Stratum I	QMH	Bea m Trawl	
1994	Thea Foss Wty.	.020	6 / 3	Reference / T.F. Stratum III	QMH	Bea m Trawl	
1993	Hylebos Waterway	.000	12 / 12	Reference / Hylebos Waterway	Blair Wty.	Bea m Trawl	

This rigorous historical analysis goes into each variable included in the Sensitivity Index, which is roughly the proportion of sensitive species per sample. Species that are not clearly sensitive or tolerant after the historical statistical analysis are not included in the index. This means that the formal definition of the Sensitivity Index is the number or biomass of sensitive species divided by the number or biomass of sensitive plus tolerant species. The Sensitivity Index, being made up of so many variables, is very stable between 1997 and 2000 (Figure 8), despite interannual fluctuations in the abundance of individual species included in the index such as *Cancer gracilis* and Pacific tomcod.

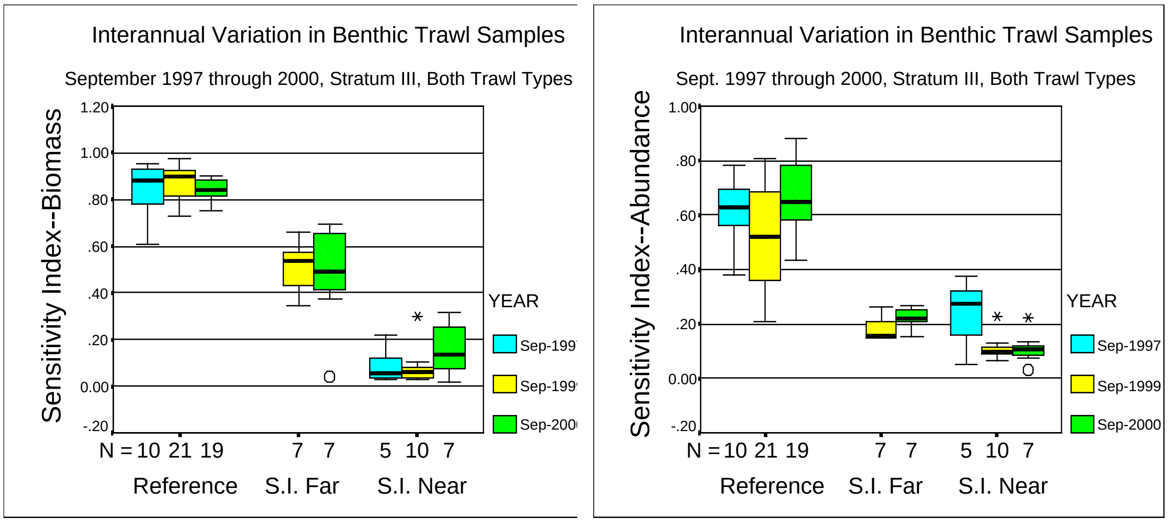

Figure 7. Sensitivity Index and Total Fish Biomass, 1997, 1999, and 2000.

Three-year interannual variation does not change the discrimination of the indices. The Sensitivity Index—Abundance shows the same consistent pattern over time as is displayed by the Sensitivity Index—Biomass, but with less (although still significant) discrimination between the farfield and nearfield sites (Figure 8).

The same composite Sensitivity Index applied to the more historical data of 1993 and 1994 shows its consistent power of discrimination for the Hylebos Waterway test site (Figure 9) and the Thea Foss Waterway test site (Figure 10). The 1993 SCCWRP otter trawl data was not significant for the abundance data (left), but the beam trawl abundance data was highly significant (right graph, p = .001). There were no biomass data available for the 1993 Hylebos Waterway versus Blair Waterway reference comparison. The Sensitivity Index (biomass) was highly significant for the Thea Foss Waterway test sites I and II versus the Quartermaster Harbor reference sites (Figure 10).

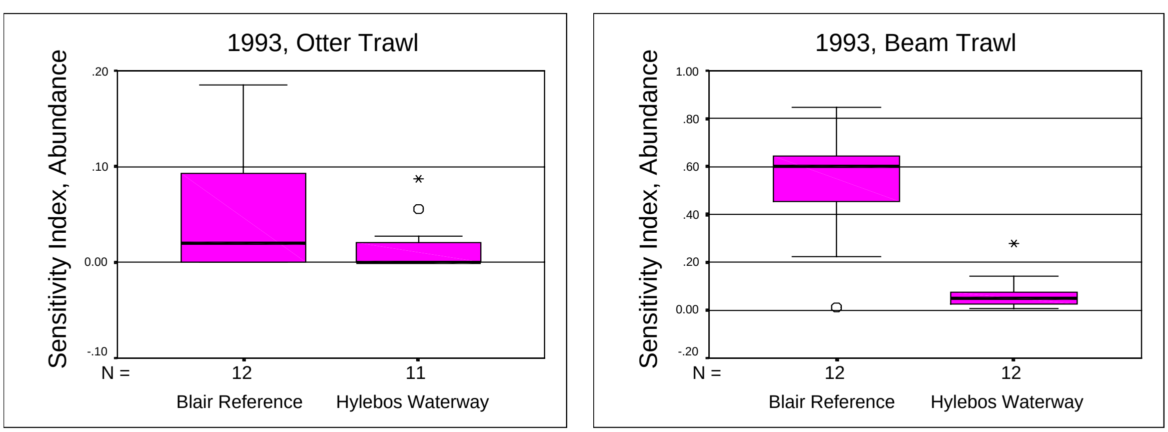

Figure 8. The 2001 Sensitivity Index applied to the 1993 trawl data from the Hylebos Waterway test site and the Blair Waterway reference site. Only the beam trawl data (right) were significantly different.

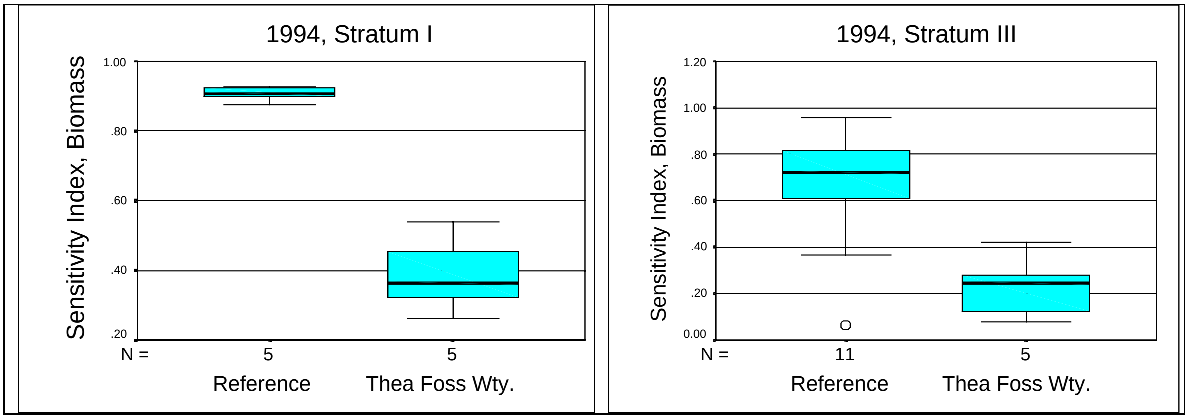

Figure 9. The 2001 Sensitivity Index applied to the 1994 trawl data from the Thea Foss Waterway test site.

Proportion of Bony Fish Biomass as Flatfish

The lower proportion of flatfish at contaminated sites (Figure 10) reflects the shift in the population away from flatfish, which live in a more constant and intimate association with the sediments, to roundfish such as tomcod, juvenile shiner surfperch, and sculpins. Other roundfish, such as the plainfin midshipman, which burrows into the sediments, feed in the water column and thus avoid the contamination of eating infaunal invertebrates. Cartilaginous roundfish such as sharks and ratfish are sensitive species and not included in the index. Their near absence from contaminated sites may help explain the increased abundance of some of the tolerant species.

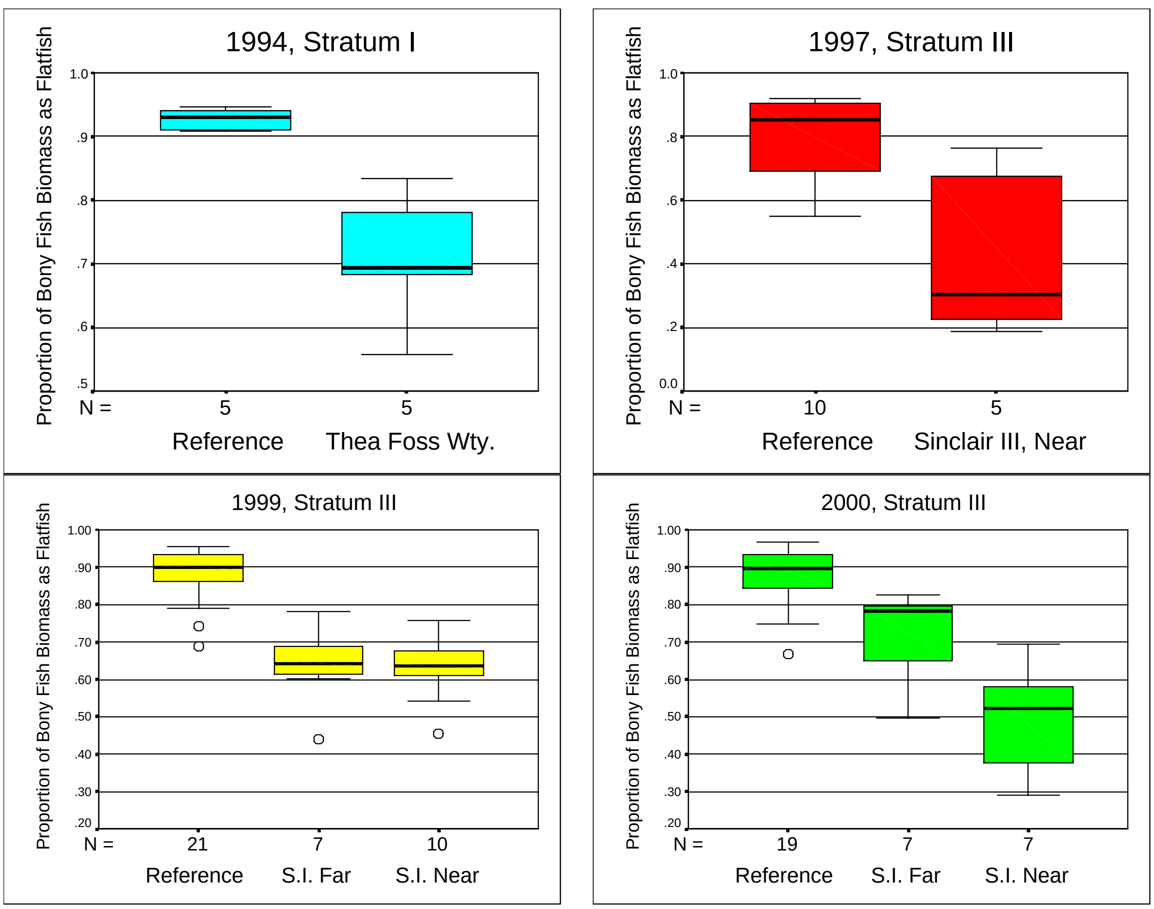

Figure 10. The proportion of bony fish biomass as flatfish usually declines with environmental stress.

Biocriteria Index Scores

Index scores are generated by comparing the population parameters (such as fish biomass) measured at the test sites against those derived from sampling reference populations living under the same physical conditions in a cleaner environment (Figure 11). The reference population acts as the benchmark—the biocriterion—for each measured parameter or metric. The number of samples (trawls) from the reference strata (N = 21) far exceeds those of any given test stratum, since the reference condition is defined from three different strata with geographic separation. The bottom of the reference box, the 25th percentile, serves as the biocriteria goal. A median of a test site, the dark line within the box, lying above this reference 25th percentile receives a score of five. The area below the reference box is divided into four equal quadrants. In the case of the graph on the left of figure 11, the Sensitivity Index for biomass, this division is easy, corresponding roughly to the horizontal lines. The median of a test site falling within the highest quadrant receives a score of 4; the next quadrant receives a three, down to the last quadrant for a score of one. The test strata arranged in descending order, with their associated scores above, include Dyes Inlet North, Dyes Inlet South, Sinclair Inlet Farfield, and Sinclair Inlet Nearfield. The graph on the right depicts the index scores for total fish biomass. In this case, Dyes Inlet North receives a score of five, since its median is above the 25th percentile (the bottom of the box) of the twenty-one reference samples represented by the box on the left of the graph.

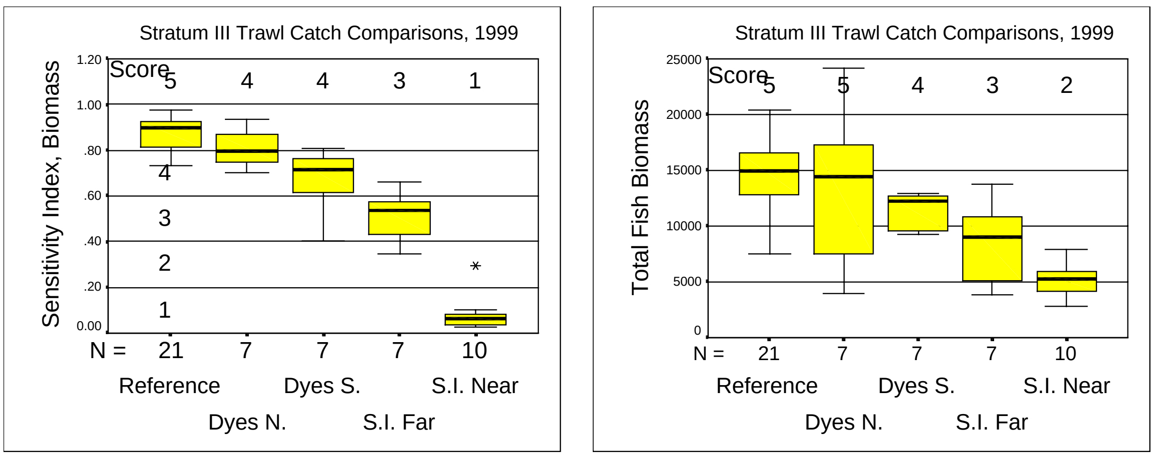

Figure 11. Index Scores for the "Sensitivity Index-Biomass" and for "Total Fish Biomass".

Total Score

Figure 12 shows the total score comprising four different metrics—total fish biomass, sensitivity index-abundance, sensitivity index-biomass, and the proportion of bony fish biomass as flatfish. There is some correlation between the sensitivity index for abundance and biomass, yet the scores are very different and seem to measure different parameters of the system. These total scores do, in fact, reflect the chemistry data from the test sites. It is also encouraging that the total index score for Sinclair Inlet is the same for 2000 as was measured in 1999 (Figure 13). Other metrics such as diversity and dominance indices are useful for other biological assemblages but were not significant as trawl metrics—corresponding more to depth and distance from the central basin than to sediment contamination. The biocriteria trawl index scores can be combined with scores from other biological assemblages, such as those derived from sampling infaunal invertebrates or fish health, to produce an even more robust score. Each of these scores would compliment the scale of the others with the largest scale derived from fish health studies, and the smallest scale from invertebrate infaunal sampling. The trawl metrics represent the intermediate scale, with its clear differentiation between the nearfield and farfield strata.

Since all progress is incremental, a first step in recovery could be accomplished by using the farfield condition rather than the reference as a short-term goal for initial progress (Figure 14). Raising the nearfield median to within the farfield box would be an encouraging first step.

Table 2. Total scores for test sites as defined by the reference strata

Stratum III, 1999

Reference Strata	Dyes Inlet North	Dyes Inlet South	Sinclair Farfield	Sinclair Nearfield	
5	5	4	3	2	Total Fish Biomass
5	2	2	2	1	Sensitivity Index, Abundance
5	4	4	3	1	Sensitivity Index, Biomass
5	5	5	3	3	Proportion of Bony Fish Biomass as Flatfish
20	16	15	11	7	Total Score

Table 3. Total Score for trawl metrics in Sinclair Inlet remains unchanged between 1999 and 2000.

Total Score: Biocriteria Trawl Metrics

Stratum III, 1999 and 2000

Reference Strata	1999 Sinclair Farfield	2000 Sinclair Farfield	1999 Sinclair Nearfield	2000 Sinclair Nearfield	
5	3	3	2	2	Total Fish Biomass
5	2	1	1	1	Sensitivity Index, Abundance
5	3	3	1	1	Sensitivity Index, Biomass
5	3	4	3	3	Proportion of Bony Fish Biomass as Flatfish
20	11	11	7	7	Total Score

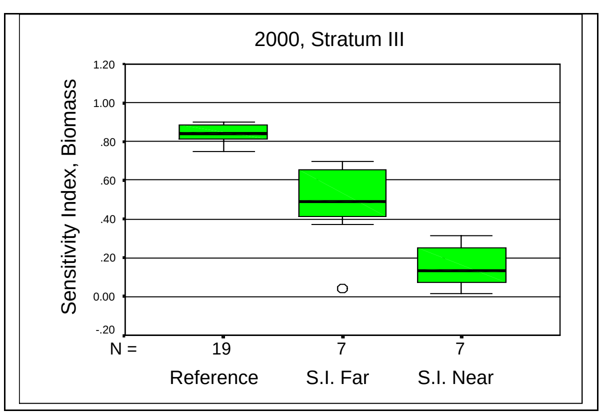

Figure 12. Farfield Stratum III in Sinclair Inlet as the initial goal for biological progress.

Discussion

Advantages of Biocriteria Trawl Metrics

The biocriteria sampling method applied to demersal trawl data has several distinct advantages for measuring environmental stress and tracking environmental recovery.

- *A measure of biological integrity*. The biological assessment process is essential to complete the intent of the Clean Water Act. The primary objective of the act, as stated in Section 101(a), is to “restore and maintain the chemical, physical, and biological integrity of the Nation’s waters.” Because the biocriteria method takes measurements directly from populations living at the test sites, it enjoys a certain ecological relevance that sediment chemistry or toxicity testing do not have.
- *Economy of sampling*. With an experienced crew, 7 to 10 trawls per day can be conducted and completely processed with very little mortality. With 5 to 7 replicate trawls per stratum, this translates to over one stratum per day.
- *A distinct sampling scale*. Sediment chemistry and infaunal invertebrate assessment essentially work on a scale on 0.10 m^2. Increasing the size of this scale requires numerous replicate samples, which, although essential in these investigations, are very expensive. Although strata can be smaller, a typical trawl stratum is 340 to 550 thousand square meters, or roughly the square footage of 100 football fields. This scale offers a uniquely integrative look at environmental stress and recovery.

Sampling Design and Strategy

- *The nearfield-farfield-reference sampling design*. This author highly recommends the incorporation of the nearfield-farfield-reference sampling design whenever physically feasible. The physical variability between nearfield and farfield strata will always be less than between nearfield and reference, allowing for a greater degree of precision. This three-tiered approach is very useful for the identification of sensitive and tolerant species, and the farfield stratum may be used as a short-term goal for initial biological recovery.
- *Sampling strategy*. Whenever possible, sampling should be rotated between strata to minimize the role of short-term variability by having all strata sampled over the same temporal period. Each stratum should be sampled over a variety of tidal cycles and times of day. One may wish to bisect the nearfield and farfield strata perpendicular to the contamination gradient before assigning random trawl stations. This stratified random design will insure that trawl stations are not clustered in the nearfield or farfield half of the stratum. The reference strata should be chosen carefully. All depths should be measured from the same baseline, e.g. Mean Lower Low Water; and temperature, salinity and dissolved oxygen should be measured twice daily to make sure that they do not exceed the defined limits. Sediment type can be measured from sediment samples using the field procedure called the “wet-sieve technique.” It is hoped that remote sediment characterization using a sonar technique is close at hand. The slope and sediment type should closely resemble that of the test sites. Sediment chemistry and toxicity should show no known impairments, and the shoreline should be as natural as possible. Some proximity of the reference sites to the test sites is necessary—both for rotational sampling and for a closer and more precise reflection of the potential biological integrity of the test sites. At the same time, some spatial variability is required to assess the full range of the reference condition. For dredged channels, it is probably unrealistic to expect them to match the index scores of a natural reference condition. A good design would be to match the test channel to one that is cleaner (e.g. Hylebos Waterway vs. Blair Waterway) and complete the sampling with a natural reference of the same physical parameters (e.g. Quartermaster Harbor). This would be a modification of the nearfield-farfield-reference design. For longer channels, such as the Duwamish Waterway, the channel could be broken up into segments displaying different levels of chemical impairment (nearfield-farfield) and compared to a cleaner reference channel such as the Snohomish River.
- *Statistical comparisons*. For the statistical comparisons between the test sites and the reference strata, the non-parametric Mann-Whitney U-test was chosen over the t-test for several reasons. Unlike the Mann-Whitney U-test, the t-test requires that all samples be normally distributed. This entails a great deal of time-consuming tests for normality—converting the data to standardized z-

scores and then analyzing for normality using either the KS or SW tests—which are not required of the non-parametric test. The second great advantage to the Mann-Whitney U-test is that it compares the ranks of the cases, thus paying attention to the medians and percentiles displayed by the box plots. Box plots are the preferred graphical representation since so much information is conveyed in a visual manner, and the metric scores are generated directly from the box plots.

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Puget Sound Research 2001

Appendix 1. Modified high-rise versus standard SCCWRP trawls.

High Rise vs. Standard SCCWRP Trawls						
	Mann Whitney Test			Mann Whitney Test		Mann Whitney Test
	Significance	Highest		Significance		Significance
No Significant Difference	(two-tailed)	Median	High Rise More Effective*	(two-tailed)	Standard More Effective*	(two-tailed)
Total fish abundance	.970	High Rise	Total invertebrate abundance	.006	Adult Snake Prickleback abundance	.020
Total fish biomass	.821	Standard	Total invertebrate biomass	.006	Adult Snake Prickleback biomass	.004
Total flatfish abundance	.940	Standard	Cancer gracilis (crab) abundance	.003	Total Snake Prickleback abundance	.045
Total flatfish biomass	.880	High Rise	Crangon spp.(mud shrimp) abundanc	.019	Total Snake Prickleback biomass	.009
Juvenile flatfish abundance	.819	Standard	Crangon spp.(mud shrimp) biomass	.037	Juvenile Pacific Tomcod abundance	.034
Average weight of adult flatfish	.940	Same	Plume anemone abundance	.035	Total Pacific Tomcod abundance	.041
Speckled Sanddab	.676	High Rise	Bay Goby abundance	.004		
Average weight of Speckled Sanddab	.880	Standard	Bay Goby biomass	.037	Standard more Effective**	
Adult Starry Flounder abundance	.590	Standard	Total Sculpin abundance	.014	Juvenile Shiner Surfperch abundance	.095
Adult Starry Flounder biomass	.878	Standard	Total Sculpin biomass	.059	Juvenile Shiner Surfperch biomass	.075
Average Weight of Adult Starry Flounder	.302	High Rise	Staghorn Sculpin abundance	.008		
Adult English Sole abundance	.760	Standard	Staghorn Sculpin biomass	.028		
Adult English Sole biomass	.364	Standard	Seastar abundance	.050		
Juvenile English Sole abundance	.908	High Rise	Seastar biomass	.045		
Adult Rock Sole abundance	.546	Standard			*Significance = $p < 0.05$	
Adult Rock Sole biomass	.579	Standard	High Rise More Effective**		**Significance = $p < 0.10$	
Average weight of adult Rock Sole	.855	Standard	Total nudibranch biomass	.081		
Juvenile Rock Sole abundance	.938	Same				
Adult Sandsole biomass	.818	Standard		Mann-Whitney non-parametric test for two independent samples		
Juvenile Sandsole biomass	.791	High Rise				
Adult Pacific Tomcod abundance	1.00	Same		N = 10 for each version of the trawl. Total N = 20.		
Adult Pacific Tomcod biomass	.597	High Rise		Two strata were sampled, upper Port Orchard and Sinclair Inlet		
Juvenile Pacific Tomcod biomass	.212	Standard		N = 5 for each version of the trawl per stratum		
Average weight of adult Pacific Tomcod	.908	High Rise		All stations randomly located (Excel random number generation)		
Pacific herring abundance	.477	Standard		Trawl types were alternately deployed		
Pacific herring biomass	.421	Standard		Statistics software = SPSS 9.0 for Windows		
Total Shiner Surfperch Abundance	.343	Standard				
Total Shiner Surfperch biomass	.850	High Rise		Both Nets:	25-foot (7.6-m.) headrope and 29-foot (8.8-m.) footline	
Adult Shiner Surfperch abundance	1.00	Same			1.5-inch body mesh of #18 nylon twine	
Juvenile Snake Prickleback abundance	.879	Standard			1.25-inch cod end mesh of #18 nylon twine	
Average weight of adult Shiner Surfperch	.307	Standard			0.25-inch knotless nylon codend liner	
Plainfin Midshipman abundance	.182	High Rise				
Plainfin Midshipman biomass	.240	High Rise		High-Rise:	Side panels open to a height of 5 feet at wing tips	
Coonstripe shrimp abundance	.500	High Rise			Footline is 0.5-inch combination poly/wire with 5.33-oz.	
Total nudibranch (sea slug) abundance	.177	High Rise			seine leads interspersed with 2-inch rubber discs	
					Headrope has eight five-inch plastic floats	
					Doors are 24-inch x 36-inch V-shaped galvanized steel	
				Standard:	Side panels open to a height of 2.5 feet at wing tips	
					Footline is 0.5-inch fiber rope with 3/16-inch galvanized	
					chain hung in loops	
					Headrope has four five-inch plastic floats	
					Doors are 20-inch x 30-inch flat wood with steel shoe	

Appendix 2. Sensitive and tolerant species determined through historical comparisons of known contaminated sites to reference sites using the Mann-Whitney U-test.

Sensitive Abundance*	Sensitive Biomass**	Tolerant Abundance and Biomass
Chondrichthyes	Chondrichthyes	Adult Rock Sole
Subadult English Sole	Subadult English Sole	Adult Sand Sole
Subadult Rock Sole	Subadult Rock Sole	Speckled Sanddab
Subadult Sand Sole	Subadult Sand Sole	Pacific Tomcod
Butter Sole	Butter Sole	White-spotted greenling
Adult Starry Flounder	Adult Starry Flounder	Subadult Shiner Surfperch
Pacific Sanddab	Pacific Sanddab	Staghorn Sculpin
Blackbelly Eelpout	Blackbelly Eelpout	Roughback Sculpin
Pacific Herring	Pacific Herring	Plainfin Midshipman
Sturgeon Poacher	Sturgeon Poacher	*Pandalus danae*
Total cucumbers	*Cucumaria piperata*	Hippolytid shrimp
Luidia foliolata	*Pentamera spp.*	Adult *Cancer gracilis*
Dendronotus spp.	*Eupentacta*	*Cancer productus*
Armina californica	*Luidia foliolata*	*Tritonia diomedea*
	Dendronotus spp.	*Solaster stimpsoni*
	Armina californica	*Pisaster brevispinus*
		Evasterias troschelii

*Giant red sea cucumber, *Parastichopus*, included in “total cucumbers”
** Giant red sea cucumber, *Parastichopus*, not included because of enormous wet weight